THE INFLUENCE OF CERTAIN FERTILIZER SALTS ON THE GROWTH AND NITROGEN - CONTENT OF SOME LEGUMES.

A THESIS

PRESENTED TO
THE FACULTY OF THE GRADUATE SCHOOL OF CORNELL UNIVERSITY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY
ALEXANDER MAC TAGGART

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Received for publication February 14, 1921

INTRODUCTION

Since the time of Hellriegel and Wilfarth (1888–90) who established the fact that symbiotic bacteria are responsible for nitrogen-assimilation by legumes, and that of Beyerinck (1888), who isolated the specific causal organism (*Bacillus radicicola*), the scientific world has believed that leguminous plants obtain the bulk of their nitrogen from the atmosphere. In recent years it has been fully demonstrated by a number of investigators also that calcium plays an important part in the soil in increasing the activity of this symbiotic organism and hence in stimulating the assimilation of nitrogen by legumes. It has, however, not been so fully shown just what fertilizing elements, other than calcium, and what combination or combinations of these elements best promote this nitrogen-assimilation and legume growth generally. To shed further light, if possible, on this somewhat obscure topic the investigation herein described was undertaken. In addition to the work of ascertaining the effect of certain fertilizer salts, containing the elements that it was thought fit to study specially, on the growth (dry-matter) and nitrogen-content of a few legumes, it was deemed advisable to investigate also the effect of these salts and of the resulting crop growth on subsequent soil nitrification.

HISTORICAL

It has been stated in the introduction to this thesis that in recent years it has been fully demonstrated that calcium plays an important part in the soil in stimulating symbiotic organisms and hence in promoting the growth of most legumes. Thus it was deemed advisable not to include lime in this investigation as a subject for further study but to provide each treatment, including that for the checks, with calcium carbonate. A mere reference here to the investigators of recent years who have found lime in various ways beneficial to legumes therefore must suffice. The list includes the following, in order according to the date of their published writings on the subject: R. Ulbricht (57); C. G. Hopkins (23); A. F. Khandurin (25); D. N. Prianisch-

nikov (46); T. L. Lyon and J. A. Bizzell (40); J. F. Duggar and M. J. Funchess (7); J. B. Abbott (1); J. G. Lipman, A. M. Blair, I. L. Owen, and H. C. McLean (36); Lipman, Blair, McLean and Wilkins, L. K. (37); Lipman and Blair (30, 31, 32, 33 34, 35); W. Frear (14); F. W. Morse (43); E. B. Fred and E. J. Graul (15, 16); H. W. Truesdell (56); J K. Wilson (60); C. R. Fellers (9). Doubtless there are other investigators who may be cited, but the experimental findings of the above-mentioned are adequate for purposes of establishing the fact that lime is beneficial in various ways to legumes as a whole.

The literature on the subject of the effect of various nutrient salts, other than calcium, on the growth and nitrogen-content of legumes, is not as extensive as that associated with lime and its effects thereon. Nevertheless, a search revealed a fair supply of published matter, particularly with reference to the action of individual elements, such as phosphorus and sulfur, on certain phases of the growth of legumes. This literature is cited below in chronological order within the citation of the published material in general appertaining to experiments with a particular nutrient element.

On the subject of the effect of nitrogen, in various forms, on the assimilation of atmospheric nitrogen and on the growth thereby of legumes, the following citations are furnished:

1910. Löhnis (39) discussed at length the earlier history of the investigations associated with this question. He cited numerous investigators and stated their individual contributions to this controversial topic, listing among the supporters of the idea of nitrogen-fixation by legumes in the presence of abundance of nitrogen, both organic and inorganic, Prazmowski, Beijerink, Frank, Böhme, Aeby, Bäszler, Nobbe and Hiltner. On the other hand there were cited the names of investigators whose work in general favored the idea of non-fixation in the presence of strong nitrates, of ammonium nitrates or sulfates, of strong accumulation of nitrogen from continuous manuring, or of water cultures. These workers included Wohltmann and Bergené, Vines, Laurent, Nobbe and Richter, Mazé, Marchall, Flamand, and Hiltner.

1914. Lipman and others (30), by means of pot experiments, showed that there was little difference in the yield and nitrogen content of soybeans fertilized with varying quantities of acid phosphate, nitrate of soda, gypsum and calcium carbonate. Gypsum gave the lowest percentage of nitrogen. Calcium carbonate, nitrate of soda, or acid phosphate in double quantity did not affect the protein content of the plant appreciably, although this increased the yield.

1915 and 1916. Lipman and Blair (31, 32, 33, 34) found that the nitrogen content of soybeans increased with applications of nitrate of soda, ammonium sulfate and dried blood. They also found that in sand cultures nodule development was not depressed by nitrogenous fertilizers, and that therewith the yield of dry matter increased up to a maximum and then decreased.

1916. Shive (52) found salts, except in weak concentrations, injurious to soybeans grown in sand. Ammonium salts, other than ammonium sulfate, exerted a more toxic action on soybeans than any of the corresponding salts of potassium, sodium and calcium.

1917. J. K. Wilson (60) pointed out the effects of various salts on nodule development. In general, chlorides, phosphates, calcium compounds and carbon-containing compounds seemed to stimulate nodule formation, while sulfates and ammonia-containing fertilizers depressed this formation on soybeans.

1917. Truesdell (56) found that the use of nitrogen did not increase the number of nodules on alfalfa roots. Nitrogen had apparently a depressing influence on the air-dry weights of the first cutting of this crop, grown in uninoculated soil, but it had no harmful effects on subsequent cuttings. He also found that the addition of nitrogen to the soil increased the total nitrogen in the roots of alfalfa.

1918. Fellers (9) showed that nitrate of soda increased the yield of soybeans but inhibited nodule formation and consequent fixation of atmospheric nitrogen, and concluded that it is not economical to supply soluble plant-food in the form of nitrogenous fertilizers to this crop. Nitrate of soda caused an appreciable increase in the protein-content of soybean seeds.

1918. Hills (22) found that the presence of large amounts of potassium, sodium, and calcium nitrates proved detrimental to the formation of nodules on alfalfa. Alfalfa seedlings grown in the presence of large amounts of nitrate did not produce nodules when inoculated with a viable culture of *B. radicicola.* Nitrates in soil cultures prevented the re-formation of nodules once removed and also caused a decrease in the number of nodules already present.

1920. Albrecht (2) concluded from his investigations that nitrogen fixation will take place in a soil containing large amounts of nitrogen in the form of either nitrates or organic matter, that no injurious effects on nitrogen fixation are caused by nitrates, that nodules are produced in the presence of large amounts of organic matter, and that variations in total nitrogen of a soil fail to affect nitrogen fixation.

On the effect of phosphorus upon the legume phenomenon, the following citations may be made:

1916. Shive (52), growing soybeans in solutions, found that phosphates caused injury to most of the seedlings where high concentrations of the radical PO_4 were employed.

1917. Truesdell (56) concluded that a part of the benefit to higher plants from phosphorus was due to some additional factor other than cellular stimulation and the quickening of soil bacterial processes, as suggested previously by Fred and Hart (17) and Lipman (29).

Working with Miami silt loam in earthenware jars under greenhouse conditions, Truesdell grew alfalfa with phosphorus (dicalcium phosphate) and phosphorus plus nitrogen (urea). The beneficial effect of phosphorus on

plant growth was noted almost from the start, and this rapid early growth may be accounted for, according to the author, only as a result of direct nutrition and stimulation of the plant by phosphorus, and as a result of the quickening of bacterial actions other than those connected with nitrogen fixation.

Phosphorus increased the formation of nodules, and this finding substantiated the previous investigations of Marchal (4), Laurent (26), Wohltmann and Bergené (61), Löhnis (38), Deherain and Demoussy (6), Flamand (10), Prucha (47), and J. K. Wilson (59).

Analyses of the roots of alfalfa showed an increase in the total nitrogen-content due to the addition of phosphorus.

The percentage of nitrogen in the first alfalfa cutting varied in inverse proportion with the dry-weights, this being in agreement with numerous observations that rapid-growing plants contain a smaller percentage of nitrogen, on dry-weight basis, than slow-growing plants. But here, though the phosphorus-treated plants grew faster than the controls, yet the total nitrogen was greater in this phosphorus-treated alfalfa.

Analyses of the third cutting, which was deemed more representative of the normal mature growth of the crop, showed an entire agreement between the results, due to phosphorus, obtained from the whole crop and from the first cutting, by way of increased total nitrogen and increased dry weight. But the third cutting showed an increase in the percentage of nitrogen in the tops for phosphorus treatment. Phosphorus caused a greater total of nitrogen and a greater percentage of nitrogen to be stored in the tops than did nitrogen treatment of the soil. The data for the inoculated and the uninoculated series agreed throughout. Consequently, the author concluded that the difference in the percentage of nitrogen must unquestionably be considered as resulting from phosphorus treatment. The results obtained by the use of phosphorus were (a) increased growth, and (b) greater efficiency in fixing and storing nitrogen. The nodule bacteria apparently had not only supplied more nitrogen to those plants that received heavier treatments of phosphorus, but had also stored a larger percentage of nitrogen in their tops.

The data seemed to indicate increased activity of root bacteria due to phosphorus, resulting in the above-mentioned benefits. This relation was especially evident in the third cutting where an additional benefit from phosphorus was expressed in the occurrence of an increased percentage of nitrogen.

1918. Fellers (9) concluded from field experiments with soybeans that the yields of total dry matter and seed are materially increased by small applications of acid phosphate, especially on well limed soils. One to two hundred pounds appeared to be as beneficial as large applications. He also found that nodule formation on soybeans was stimulated, on limed soils, by acid phosphate. The stimulation was not so marked on acid soils. This fertilizer seemed to exert a beneficial influence on protein formation in the seed on both limed and unlimed plots. The fertilizer treatment for soybeans that appeared to give the best return for the money invested was probably 200 to 400 pounds of acid phosphate, together with a ton of lime, per acre.

The intimate relation of potash to nitrogen-assimilation by legumes has in the past been definitely established by various investigators. Recent investigations on the subject of potash fertilizing of legumes however may be cited.

1918. Fellers (9), by field experiments, showed that muriate of potash in applications of 50 to 400 pounds per acre gave an average increase of about 10 per cent in the yield of total dry matter and seed of soybeans on both limed and unlimed plots. Nodule production was slightly stimulated on the limed plots but not on the unlimed. Potash, he found, had little influence on the protein content of the seeds of soybeans.

The literature on the subject of the effects of sulfur upon the growth and nitrogen content of legumes is fairly extensive, and from this published material the following citations may be made:

1911. Hart and Peterson (20) called attention to the apparent deficiency of sulfur in certain soils as related to the demands made upon this element by some species of agricultural plants, legumes included. Analyses of these crops showed alfalfa especially high in sulfur content, and that this crop's sulfur requirements were actually greater than the phosphorus requirements.

1912. Bernard (3) found crop increases from the use of sulfur.

1912. Boullanger (4) obtained increased yields of crops from the sulfur treatment of the soil.

1913. C. B. Lipman (27) concluded that gypsum stimulated the beneficial soil organisms on the roots of legumes.

1914. Lipman and Blair (30) fertilized soybeans grown in pots, with calcium sulfate, nitrate of soda and calcium carbonate at applications of 10 gm. and 25 gm. The maximum yield of soybeans for a single pot was obtained from the calcium sulfate treatment.

1914. Shedd (50) obtained beneficial effects from sulfate with various crops grown in soil cultures. There were decided gains in the growth of soybeans with applications of sulfur, ammonium sulfate, pyrite and ferrous sulfate and smaller gains with calcium, potassium, barium, magnesium, aluminum and sodium sulfates on a soil containing 600 pounds of sulfur and 3040 pounds of phosphorus per acre.

1914. Reimer (48) obtained increased yields of alfalfa grown in the presence of flowers of sulfur.

1915. Hart and Tottingham (21), by means of soil cultures in the greenhouse, found that sulfur in the form of calcium sulfate, more so than in the form of sodium sulfate, was beneficial to common red clover, especially lengthening its root-system, hence feeding power, and increasing the yield of the dry matter 23 per cent. They showed also increased yields of legumes with calcium sulfate added to a complete fertilizer over a complete fertilizer plus potassium chloride. Here, they claimed, the action of the calcium sulfate must have been direct.

The same investigators found that calcium sulfate was especially favorable in increasing the yield of grain in peas. Its effect in increasing straw was more in evidence with beans and red clover.

1916. Pitz (45) concluded that calcium sulfate in small amounts increased the yield of red clover and the formation of nodules. Sulfates stimulated the development of red clover bacteria as well as the young plant. Elemental sulfur, however, increased the yield of red clover but slightly, and did not affect the root development nor the formation of nodules.

1916. Duley (8) found that when used alone on silt loam soil, flowers of sulfur was beneficial to the yield of red clover. It also very markedly increased nodule production on the roots of red clover when added to a complete fertilizer.

1917. Shedd (51) grew soybeans, red clover, alfalfa, and other legumes with 100 to 200 pounds of flowers of sulfur. He found that in the soybeans, which showed an increased sulfur content, no corresponding increased protein content always was found. In five out of eight instances, however, soybeans grown in soil where sulfur was added showed an increase in the total weight of protein.

1917. Brown (5), from experiments conducted in the Hood River Valley of southern Oregon, states that sulfur is a valuable fertilizer for alfalfa, the sulfur content of which is very high, according to the experiment station analyses. There air-slacked lime failed to produce increased yields of alfalfa, but when followed by a 100-pound application of land plaster (calcium sulfate) at the end of the first cutting, the plants immediately took on renewed vigor and easily surpassed the unfertilized plot on a total season's yield by the end of the last, or third cutting. This increase was shown despite the fact that the first cutting showed 1168 pounds for the check versus only 480 pounds for the other. The experiments with flowers of sulfur did not show such large increases of alfalfa, and it would seem, stated the author, that the lighter applications are the most economical when applied each year. Sulfur being quite insoluble in water, hence not immediately available, it was recommended that it be applied in the fall or not later than January or February, whereas land plaster should be applied as early as March to produce good results.

1918. Tottingham (55) showed that the addition of sodium sulfate and calcium sulfate to the sulfur-free modification of Knop's solution, in amounts equivalent to the sulfur of the unmodified solution, produced a greater yield of dry tops of red clover than did the latter solution, calcium sulfate being very efficient in this respect. It appeared as if the sulfur of gypsum functioned in the molecular combination in which it was supplied. The data obtained indicated that a deficiency of sulfur supply restricts growth by limiting the synthesis of protein. The author stated that the more or less parallel fluctuations of the plane of sulfur supply, the weight of nitrogen assimilated, and the yield of dry tops of the red clover plants, indicated that sulfur deficiency restricted growth by limiting this synthesis of protein.

1919. Miller (42) concluded that the great increase found in the nitrogen content of the clover grown in soil where sulfate had been added, is the result, in all probability, of these sulfates stimulating the action of legume bacteria.

His experiments also showed that sulfates caused an increase in root development and in the number of nodules on the red clover roots.

1919. Reimer and Tartar (49) found that on various types of soil alfalfa and red clover were increased from 50 to 1000 per cent by the use of various types of fertilizers containing sulfur, gypsum included. The soils ranged from coarse granite soils to the heaviest adobes. None were acid nor noticeably alkaline. Fall applications gave best results. The sulfur fertilizers used were very stimulative of the root system, increasing its size and the number of nodules. The fertilized plants contained more sulfur, more protein, and more nitrogen than the unfertilized. Gypsum was equal to superphosphate in results, but it was expected that eventually the latter would give superior returns, because the phosphorus content of the soils experimented with was rather low. Rock phosphate gave negative results in this region.

1920. Stewart (54), from very slight increases in the yield of soybeans and alfalfa grown in the field, and from slight decreases in clover yields, over a period of years, concluded that sulfur is not a factor in the production of crops, on brown silt loam at least. After examining the results obtained with gypsum during a period of 18 years at the Ohio station, he concluded that it is quite evident that the apparently beneficial action of gypsum is due to its stimulating effect, particularly on bacterial life (shown by Greaves), thus enabling the crop to draw better upon the inadequate supply of phosphorus in the soil.

1920. Singh (53) found, by the use of pot cultures, that gypsum generally increased the process of fixation of nitrogen by *B. radicicola*, the greatest increase occurring with the largest application. He further found that 1000 pounds of gypsum increased the yield of red clover, but that other applications did not have any effect on other legumes (alfalfa, Canada field peas, and soybeans). The nitrogen content of legumes, he found, was not affected by gypsum.

The literature upon the subject of the effect of fertilizer salts upon soil nitrification appears to be somewhat limited. A few citations having a bearing upon this phase of our investigation however may be stated.

1904. Fraps (11) pointed out that phosphoric acid and potash increased nitrification in some soils, while in other soils the opposite effect was produced.

1908. The same investigator (12) showed that these soil constituents had little effect upon the production of active nitrogen, though in some cases nitrification was affected considerably. With both phosphoric acid and potash the active nitrogen was much less affected than the production of nitrates.

1920. Fraps (13) also found that the addition of phosphate and of potash to potted soils increased nitrification in several types of soil and caused the soils which nitrify very slowly to nitrify in a shorter time. Dicalcium phosphate was more effective than potash (K_2SO_4) in these respects. He further showed that calcium carbonate increased nitrification. During these experiments, however, a considerable time elapsed before he noticed the formation of nitrates.

1909. Lipman (28) observed that the amounts of NO_3 nitrogen in parts per million were favorably affected by gypsum.

1912. Patterson and Scott (44) found that superphosphate increased nitrification of ammonia added to a soil, and concluded that this fertilizer may prove a useful aid to nitrification. The soil, however, was poor in P_2O_5 (0.032 per cent). They suggested that phosphates may help to nourish nitrifying organisms as well as the crop; and that where not required by these organisms, superphosphate, being acid, will probably do harm. Gypsum, they found, had a moderate effect in encouraging nitrification, but was not at all equal to calcium carbonate in this respect. They further showed that sodium chloride (salt) had a bad all-round effect on nitrate production.

1916. Jensen (24) found that bone meal, superphosphate, waste lime, and dry yard manure decreased the nitrifying activity in field soils. The manured plots lost most nitrogen, especially those to which ammonium sulfate was added, while the limed plots showed a gain in total nitrogen. Plots receiving calcium cyanamid, phosphatic fertilizers, and nitrate showed a slight gain in total nitrogen over the checks.

1916. Duley (8) showed that the nitrate content of the soil varied inversely with the amount of soluble sulfate in the soil.

1918. Fulmer (18) found that while nitrification is benefited by limestone, calcium carbonate and magnesium carbonate (particularly by the latter), it is only very slightly increased by phosphates (dibasic magnesium phosphate and monocalcium phosphate were used) in certain Wisconsin soils.

1918. Greaves (19) and his co-workers showed that calcium sulfate is more efficient than potassium chloride as a stimulator of nitrification, increasing nitric-nitrogen accumulation of the soil 97 per cent. They found that those compounds which are the strongest plant stimulants also are the most active in increasing nitric-nitrogen accumulation of the soil, and that it is very likely that the effect upon the plant is due mainly to the action of the compound upon the bacteria, which in turn render available more plant-food. They asserted, however, that the ammonifying powers of a soil containing alkalis are a better index to its crop-producing powers than are the nitrifying powers. They further found that nitrification was least with KCl out of the six chlorides experimented with. The soil, however, contained over 7 per cent of $CaCO_3$, and therefore was suited for satisfactory nitrification results from the use of gypsum.

1920. Whiting and Schoonover (58), working with field soils in which soybeans were grown, showed that phosphorus in the form of rock phosphate increased nitric nitrogen to the extent of 18.09 to 19.01 pounds per acre, over and above that produced by organic matter (stable manure or crop residues).

1920. Singh (53), working with pot cultures, found that nitrification was depressed by gypsum alone, but the use of gypsum and lime together increased the process.

EXPERIMENTAL

Methods and results

Thirty-six square, stout wooden boxes were each filled with 128 pounds of a mixture composed of 110 pounds of clean sand and 18 pounds of a sandy loam soil. The soil medium was thus decidedly low in plant nutrients but contained enough to supply the crops grown provided it was in an available condition. This was designed to make very pronounced the effect of those fertilizer nutrients in the soil that were not readily available as compared with those that were. The inclusion of the loam served the purpose of introducing the nitrifying organisms. The subsequent crop growth was carried out in the greenhouse. The content of each box was compacted alike, and the moisture content of the soil, as far as possible, was maintained throughout at 10 per cent (on the dry-soil basis) by weighing the boxes at regular intervals, varying with the crop and with the stage of the growing season. On November 7 and 9, respectively, alfalfa and Canada field peas were each sown in 18 boxes containing 9 separate treatments, in duplicate. To each box was added $\frac{3}{4}$ pound of calcium carbonate, it having been shown by various investigators to promote assimilation of nitrogen by legumes. The varying treatments were as follows:

BOX NUMBER	TREATMENT
1, 10, 19, 28	No fertilizer (checks)
2, 11, 20, 29	Nitrogen (dried blood, 12 gm. per box)
3, 12, 21, 30	Phosphorus (disodium phosphate, 8 gm. per box)
4, 13, 22, 31	Potassium (muriate of potash, 8 gm. per box)
5, 14, 23, 32	Sulfur (gypsum, 8 gm. per box)
6, 15, 24, 33	Nitrogen, phosphorus, potash and sulfur in above forms (total 36 gm.)
7, 16, 25, 34	Nitrogen, phosphorus, and potash in above forms (total 28 gm.)
8, 17, 26, 35	Nitrogen, potash, and sulfur, in above forms (total 28 gm.)
9, 18, 27, 36	Phosphorus, potash, and sulfur, in above forms (total 24 gm.)

Previous to seeding, the boxes were inoculated with sand cultures containing the sub-species of *B. radicicola* corresponding to the legume sown. In boxes 1 to 18 alfalfa was sown at the same rate as ordinarily sown under field conditions. The plants were subsequently thinned out to 23 per box. Boxes 19 to 36 were seeded with Canada field peas at the rate of 25 per box. These were later thinned out to 11 per box.

Because of backwardness in becoming established, due doubtless to an insufficient supply of nitrogen, the alfalfa seedlings were sprinkled on January 23, 1920, with a solution of nitrate of soda at the rate of 1.94 gm. per box (approximately 100 pounds per acre of 3,000,000 pounds of soil).

Five cuttings of alfalfa (cut when almost fully flowered, except in the case of cutting no. 5 which failed to flower because of the lateness and coolness of the season) were obtained. These were dried in the drying chamber, weighed and analyzed for dry matter and total nitrogen.

The peas, which produced an enormous growth, were carefully kept upright, and were harvested when fully ripe. The grain and straw were weighed, and analyzed for dry-matter and nitrogen, separately. Photographs of the pea growth are shown in plate 1.

Following the crop of Canada field peas, Ito San soybeans were seeded on May 22, 1920, after suitable inoculation of the soil. These were kept upright also and allowed to ripen fully before harvesting. The grain and straw were weighed, and separately analyzed for dry matter and nitrogen.

The lime, in the form of calcium carbonate, was applied at the rate of 3 tons (of 2000 pounds) per acre of 3,000,000 pounds of soil, the salts at the rate of 282.6 pounds per acre, and the dried blood at the rate of 424 pounds per acre.

TABLE 1

Average total dry matter in the various crops for the various treatments

TREATMENT	ALFALFA (23 PLANTS, TOTAL OF 5 CUTTINGS)	CANADA FIELD PEAS (11 PLANTS)			SOYBEANS (12 PLANTS)		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Lime alone (check)	157.200	42.117	62.63	104.750	30.520	72.825	103.345
Lime and nitrogen	154.935	53.480	74.49	127.970	30.970	74.525	105.495
Lime and phosphorus	192.805	72.400	123.67	196.075	33.260*	88.920	122.180
Lime and potassium	163.490	50.217	72.36	122.580	29.735	69.635	99.370
Lime and sulfur	168.665	25.140	49.85	75.000	30.635	71.600	102.235
Lime, nitrogen, phosphorus, potassium and sulfur	209.925	77.665	125.40	203.070	35.435	86.165	121.600
Lime, nitrogen, phosphorus and potassium	197.690	77.250	130.62	207.870	32.205	85.940	118.145
Lime, nitrogen, potassium and sulfur	174.995	32.677	56.54	89.220	28.520	73.135	101.655
Lime, phosphorus, potassium, and sulfur	186.290	75.370	128.33	203.705	34.230	82.465	116.695

* Only one box included in average.

Following the harvesting of the soybeans and of the fifth cutting of alfalfa, the boxes of soil (plus roots) were incubated for three weeks at greenhouse temperatures, the moisture content at 10 per cent being maintained throughout this period. Immediately following incubation, the contents of the boxes were carefully sampled by making six full-depth borings with a soil auger in each box. These samples were immediately extracted with distilled water and the extracts analyzed for nitrate nitrogen by the colorimetric method.

In the determination of total nitrogen in the various crops the Kjeldahl-Gunning method was used throughout. These determinations were conducted for the most part in duplicate, but where wide or reasonably wide variations between the duplicates occurred (as happened in a few instances, especially in analyzing the grain) triplicate determinations were made and the

nearest two titrations were selected for averaging. The dry-matter and nitrate-nitrogen determinations also were conducted in duplicate.

Tables 1, 2 and 3 show the average weights of dry matter and of the total nitrogen, also the average nitrogen percentages (based on the dry matter) for the duplicate boxes growing the three crops under all treatments.

Table 4 gives the averaged nitrification results from all salts, including lime (checks), after the growth of crops.

Tables 5, 6 and 7 record the percentage increases of dry matter, of total nitrogen, and of the percentage of nitrogen in the three legumes as the result of soil treatment with the above-mentioned nutrient salts. From these tables and tables 8 and 9 the conclusions enumerated at the close of this thesis have been drawn.

TABLE 2

Average total nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUTTINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Lime alone (check)	5.300	1.895	0.605	2.500	2.170	0.910	3.080
Lime and nitrogen	5.315	2.480	1.010	3.490	2.340	0.890	3.230
Lime and phosphorus	6.925	3.450	1.705	5.155	2.690	1.550	4.240
Lime and potassium	5.700	2.750	1.110	3.860	2.210	0.810	3.020
Lime and sulfur	5.645	1.235	0.860	2.095	2.240	0.815	3.055
Lime, nitrogen, phosphorus, potassium and sulfur	7.335	3.645	1.740	5.385	2.605	1.390	3.995
Lime, nitrogen, phosphorus and potassium	7.065	3.780	2.090	5.870	2.520	1.460	3.980
Lime, nitrogen, potassium and sulfur	5.930	1.560	1.055	2.615	2.105	0.995	3.100
Lime, phosphorus, potassium and sulfur	6.570	3.470	2.230	5.700	2.585	1.225	3.810

Tables 8 and 9 show the actual and percentage increases of nitrate nitrogen, in parts per million, after the growth of alfalfa and of Canada field peas and soybeans by the various nutrient salts. Dry-matter increases (actual) also are included for comparison with the corresponding nitrate-nitrogen increases.

During the growth of the legumes a few notes of special interest respecting the behavior of the plants were made from time to time.

In the peas the potash-treated plants were the first to flower, blossoms being noticed on the tall phosphorus-treated plants some two days later. Where potash was supplied the pods appeared to be best filled, while plants without a potash supply seemed insufficiently filled. Where a complete fertilizer was added the pods were more advanced and the vines ripened before those in the other boxes.

In the alfalfa the plants that received phosphorus flowered first and thereon the flowers were the most abundant. The accelerating effect of phosphorus on the reproductive parts of the crop was here demonstrated.

In the first growth of alfalfa an apparently injurious effect of sulfur was somewhat noticeable, but in later cuttings this was not visible. The inhibiting action on growth, more especially where sulfur was used alone, had disappeared, as is recorded in the percentage increases for the second and subsequent cuttings. On the other hand, this effect of sulfur used alone on the peas was visible throughout the growth of the crop.

TABLE 3

Average percentage of nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUTTINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lime alone (check)	3.420	4.480	0.960	2.720	7.125	1.240	4.18
Lime and nitrogen	3.490	4.640	1.360	3.000	7.525	1.200	4.36
Lime and phosphorus	3.616	4.765	1.385	3.075	8.090	1.750	4.92
Lime and potassium	3.559	4.530	1.535	3.032	7.430	1.160	4.29
Lime and sulfur	3.415	4.825	1.795	3.310	7.305	1.145	4.22
Lime, nitrogen, phosphorus, potassium and sulfur	3.555	4.695	1.360	3.027	7.335	1.610	4.47
Lime, nitrogen, phosphorus and potassium	3.611	4.890	1.610	3.250	7.845	1.710	4.78
Lime, nitrogen, potassium and sulfur	3.479	4.825	1.870	3.347	7.375	1.355	4.36
Lime, phosphorus, potassium and sulfur	3.600	4.600	1.735	3.167	7.555	1.485	4.52

TABLE 4

Average nitrate nitrogen in the soil after removal of crops, for the various treatments

TREATMENT	NO_3 IN DRY SOIL		REMARKS
	After alfalfa	After Canada field peas and soybeans	
	p. p. m.	*p. p. m.*	
Lime alone (checks)	4.95	10.7	Nitrification determinations after Canada field peas and soybeans, grown successively, had the advantage of the well rotted root system of the pea crop
Lime and nitrogen	6.85	11.4	
Lime and phosphorus	10.85	13.2	
Lime and potassium	4.50	7.4	
Lime and sulfur	5.85	8.2	
Lime, nitrogen, phosphorus, potassium, and sulfur	8.80	15.7	
Lime, nitrogen, phosphorus and potassium	13.65	22.5	
Lime, nitrogen, potassium, and sulfur	11.55	10.5	
Lime, phosphorus, potassium and sulfur	8.55	18.0	

Discussion of crop results

Upon referring to tables 1 to 3 and 5 to 7, it will be at once noticed that phosphorus has produced the most marked effect of all of the elements applied. The effect of phosphorus in increasing the dry matter, total nitrogen, and also, although to a lesser extent, the percentage of nitrogen in the legumes grown, is unmistakable. The literature cited above substantiates these findings with respect to the beneficial influence of phosphorus; and these results add further testimony to the importance of this vital substance to the growth of crops and to the growth of leguminous crops in particular. Doubtless, this decidedly beneficial influence is due mainly to the bacterial stimulus by phosphorus, as is indicated by Truesdell (56).

TABLE 5

Percentage increases of total dry matter over the checks, due to various treatments, for the three legumes*

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen	−1.504	22.167	2.080
Phosphorus	22.571	87.183	18.225
Potassium	3.935	17.021	−3.847
Sulfur	7.225	−28.401	−1.074
Nitrogen, phosphorus, potassium and sulfur	33.455	93.861	17.664
Nitrogen, phosphorus and potassium	25.677	98.443	14.321
Nitrogen, potassium and sulfur	11.249	−14.826	−1.636
Phosphorus, potassium and sulfur	18.429	94.467	12.917

* Checks received lime alone and all treatments contained lime at the same rate.

TABLE 6

Percentage increases of total nitrogen over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen	0.283	39.6	4.870
Phosphorus	30.660	106.2	37.662
Potassium	7.547	54.4	−1.948
Sulfur	6.509	−16.2	−0.812
Nitrogen, phosphorus, potassium and sulfur	38.396	115.4	29.707
Nitrogen, phosphorus and potassium	33.301	134.8	29.202
Nitrogen, potassium and sulfur	11.886	4.6	0.649
Phosphorus, potassium and sulfur	23.962	128.0	23.701

With legumes, this experiment has indicated that any fertilizer, possibly with the exception of sulfur, that increases yield increases the percentage of nitrogen.

Naturally, combined nitrogen is not as essential to legumes as is phosphorus. Nevertheless, we find it playing some part in the growth of these crops, varying with the crop and its habit of growth and with the association of elements in which nitrogen is employed. For example, of the three plant species, peas were benefited in growth the most by nitrogen when it was used alone, while alfalfa was the least benefited; whereas by nitrogen in combination with other substances alfalfa was benefited in growth the most, and peas the least. While nitrogen, used alone, here slightly increased the percentage of nitrogen in the three legumes, particularly in the case of peas, yet when used in combination with other substances it did not have this effect.

In general, combined nitrogen in this experiment appeared to play some part in promoting nitrogen assimilation by legumes. It at least did not hamper the operation of this phenomenon, in keeping with the findings of the

TABLE 7

Percentage increases of percentage of nitrogen in plants over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (AVERAGE OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen	2.046	10.294	4.306
Phosphorus	5.731	13.051	17.703
Potassium	4.064	11.489	2.631
Sulfur	−0.146	21.691	0.957
Nitrogen, phosphorus, potassium and sulfur	3.947	11.305	6.937
Nitrogen, phosphorus and potassium	5.584	19.485	14.354
Nitrogen, potassium and sulfur	1.725	23.069	4.306
Phosphorus, potassium and sulfur	5.263	16.452	8.134

majority of the investigators cited under this section; but whether or not the action would be impaired in the presence of large quantities of nitrogen is not within the scope of this investigation to answer.

The treatments were so arranged that only the effects of potassium used alone can be considered and these effects are beneficial in the cases of the growth of peas and of alfalfa, but apparently not in the case of the growth of soybeans. Peas were the most benefited in growth by muriate of potash, for this crop, of all three crops, showed the largest percentage increases of dry matter, of total nitrogen, and of percentage of nitrogen with potassium treatment.

Sulfur, without other fertilizer substances and in the form of gypsum, was apparently toxic to peas and slightly toxic to soybeans. To alfalfa, however, it proved beneficial, and this effect increased with the development of the crop, as shown by the successive cuttings, doubtless because of the disappearance of the toxic influence at first established in the soil. Had a less sandy soil been used the seemingly toxic effect noted, in all probability, would have

been less in evidence. Sulfur in combination with other substances was apparently toxic only in the case of peas, and even here this seeming toxicity was less marked than it was where sulfur was used alone. The fact that the treatments contained lime in fair quantity may possibly have accounted, in no small measure, for the satisfactory results obtained with alfalfa when fertilized with calcium sulfate—an experience recorded from experiments embodying the use of gypsum on calcareous soils.

As shown by Truesdell (56) in his investigations, the third cutting of alfalfa, on the whole, was the most satisfactory, the yields of dry-matter and the analyses being in general higher than those associated with the other cuttings.

The striking differences for the various treatments shown throughout the investigation have been made possible as the result mainly of using a compounded soil that was practically a sand. Had an ordinary soil been used, these differences would in large measure have been masked by the effect of plant-food elements inherent in the soil. The results herein obtained can at least lay claim to have in some small measure strengthened our knowledge of the growth requirements of legumes, and of alfalfa, Canada field peas and soybeans in particular.

TABLE 8

Increase in soil nitrification due to salts, after growth of alfalfa (5 cuttings)

INCREASE DUE TO	INCREASE OF NO_3 OVER CHECK		INCREASE OF TOTAL DRY MATTER
	p. p. m.	*per cent*	*gm.*
Nitrogen	1.90	38.38	−2.365
Phosphorus	5.90	98.98	35.505
Potassium	−0.45	−9.09	6.190
Sulfur	0.90	18.18	11.365
Nitrogen, phosphorus, potassium and sulfur	3.85	77.77	52.625
Nitrogen, phosphorus and potassium	8.70	175.75	40.390
Nitrogen, potassium and sulfur	6.60	133.33	17.695
Phosphorus, potassium and sulfur	3.60	72.72	28.990

TABLE 9

Increase in soil nitrification due to salts, after growth of Canada field peas and soybeans

INCREASE DUE TO	INCREASE OF NO_3 OVER CHECK		INCREASE OF TOTAL DRY MATTER	
			Peas and soybeans	Peas alone
	p. p. m.	*per cent*	*gm.*	*gm.*
Nitrogen	0.7	6.54	25.370	23.220
Phosphorus	2.5	23.36	110.160	91.325
Potassium	−3.0	−28.03	13.855	17.830
Sulfur	−2.5	−23.36	−30.860	−29.750
Nitrogen, phosphorus, potassium and sulfur	5.0	46.72	116.575	75.100
Nitrogen, phosphorus and potassium	11.8	110.28	117.920	103.120
Nitrogen, potassium and sulfur	−0.2	−1.87	−17.220	−15.530
Phosphorus, potassium and sulfur	7.3	68.22	112.305	98.955

Discussion of soil nitrification results

A perusal of the soil nitrification results, as recorded in tables 4, 8, and 9, shows that salts or their combinations which most markedly promoted the growth of legumes usually caused the highest nitrification. Such was particularly the case wherever phosphorus was applied. This observation concurs with the conclusion of Greaves (19) who found that those compounds which are the strongest plant stimulants are also the most active in increasing nitric-nitrogen accumulation in the soil. He attributes this correlation to the stimulus given to the bacteria by the beneficial compound. This may be a factor in the results herein recorded, but we are inclined to give some recognition also to the effect of the decayed roots of the previous crop upon nitric-nitrogen accumulation. The increased top growth is correlated with increased root development, hence with more organic matter for nitrification. There was greater nitrification after peas and soybeans (grown in the same boxes of soil) than after alfalfa (five cuttings). The extensive root systems of the huge pea plants had opportunity to decay well, whereas there would be less decay of the alfalfa roots, even though extensive.

Nitrogen, applied alone, increased soil nitrification after all three crops, particularly after alfalfa; but when this nutrient was applied in combination with the other substances, it decreased nitrification after peas and soybeans and slightly increased it after alfalfa. It would thus appear that alfalfa is less dependent upon nitrate nitrogen for growth than are the other two legumes, peas especially.

Sulfur depressed nitrate-nitrogen accumulation, except when used alone as a fertilizer nutrient for alfalfa, which crop it also otherwise benefited, both alone and combined with other elements. In general, this finding was in accordance with the findings of Duley (8) who found that the nitrate-content of the soil varied inversely with the amount of soluble sulfate in the soil.

Potassium apparently slightly inhibited nitrate-accumulation after all three crops. It may here be mentioned, however, that because of the presence of chlorides (in the KCl used) there may possibly have been a slight loss of nitrates during the process of determination by the colorimetric method, which involves the use of pheno-disulfonic acid.

CONCLUSIONS

Effects of phosphorus

Of all the fertilizer elements in the salts applied to the compounded soil, phosphorus showed the most marked effect. As a single element it markedly increased the dry-matter and total nitrogen, and to a lesser extent the percentage of nitrogen in all three legumes, the order of greatest average influence on the crop being: (a) Canada field peas, (b) soybeans and (c) alfalfa. In the three crops phosphorus, used alone, showed its powerful influence on the three

factors in the following order: (a) increase of total nitrogen; (b) increase of dry-matter and (c) increase in the percentage of nitrogen.

In combination with nitrogen, potassium and sulfur, phosphorus markedly increased the dry matter and total nitrogen in Canada field peas, soybeans and alfalfa. However, it increased the percentage of nitrogen in soybeans and alfalfa only slightly, if at all, and decreased the percentage in the case of peas.

Effects of nitrogen

As a single element nitrogen can hardly be said to benefit the plants with respect to yields of either dry matter or nitrogen, or the percentage of nitrogen, unless in the case of Canada field peas, which appeared to respond somewhat in each of these three properties.

In combination with phosphorus, potassium, and sulfur, nitrogen promoted no more response in the legumes than where it was employed alone. Indeed, there was perhaps less response from nitrogen when used in association with the other elements.

Combined nitrogen did not hamper the operation of nitrogen assimilation by legumes; but whether or not it would have hindered the phenomenon had large quantities of nitrogen been used, could not be answered by this experiment.

Effects of potassium

Potassium, used alone, showed its greatest influence in increasing, on the average, the total nitrogen and dry matter in Canada field peas and alfalfa, in the order named. In soybeans, however, it showed a decrease with respect to these factors. Only in the percentage of nitrogen did potassium show an increase common to all three crops, and this in the crop order just named.

Effects of sulfur

Sulfur, in the form of gypsum, used alone and in combination with other fertilizer salts, increased somewhat the growth and nitrogen content of alfalfa but appears not to have had any effect on field peas and soybeans.

General effect of fertilizer salts

In general it may be said that when any application of fertilizer, with the exception of gypsum, increased the yield of the legumes grown, there was also an increase in the percentage of nitrogen in the plants.

Effects of fertilizer salts on soil nitrification, after legumes

Where phosphorus was applied there was, in general, the greatest nitrate accumulation after all crops. Thus salts or their combinations which most markedly promoted the growth of legumes, as did phosphorus, usually caused the greatest nitrification.

Nitrogen applied alone increased soil nitrification after all three crops, particularly after alfalfa, but when this nutrient was applied in combination with the other substances it did not have such an effect.

Potassium, in the form of muriate of potash, apparently slightly inhibited nitrate-nitrogen accumulation.

Sulfur, in the form of gypsum, increased nitrification in soil in which alfalfa had grown but not in soil in which peas and soybeans had grown. There appears to be a connection between the effect of sulfur on the crop and on nitrification following the crop.

In general, there appeared to be a tendency toward correlation between the dry matter produced and subsequent soil nitrification—due in part, it is assumed, to the greater root system associated with greater top growth, hence to greater amounts of decayed roots for promoting nitrification.

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PLATE 1

FIG. 1. Canada field peas fertilized with individual elements; note the pronounced effect of phosphorus.

FIG. 2. Canada field peas fertilized with elements in various combinations; note the pronounced effect of phosphorus.

Fig. 1

Fig. 2